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(54) **Lymphographic and Organ Imaging Materials and Kit.**

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CHEMICAL ABSTRACTS, vol. 103, no. 7, August 1985, ref. no. 675347, Columbus, Ohio, US; GOODWIN et al.: "Chelate conjugates of monolonal antibodies for imaging lymphoid structure in the mouse" & J.NUCL. MED. 1985, 26(5), 493-502

(73) Proprietor: **Immunomedics, Inc.**
300 American Road
Morris Plains,
New Jersey 07950 (US)

(72) Inventor: **Goldenberg, Milton David**
397 Long Hill Drive
Short Hills, New Jersey 07078 (US)

(74) Representative: **Sheard, Andrew Gregory et al**
Kilburn & Strode
30, John Street
London WC1N 2DD (GB)

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Description

BACKGROUND OF THE INVENTION

The present invention relates to a method for imaging lymphatic structures and to a kit suitable for use therefor.

There is a need, particularly in oncology, for a method that clearly delineates lymphatic structures. Lymphatic structures, particularly lymph nodes, drain tissue and extravascular regions of various molecular and macromolecular substances, including antigens, infectious agents, and cells, serving as a filter as well as a part of the host organism's immunological apparatus. It is well known that certain substances with appropriate physical properties, when injected into a suitable tissue plane, are transported from the injection site by a drainage system and sequestered in regional and then more distant lymph nodes. Some of these substances, particularly colloids, are passively retained in sinusoids and actively phagocytosed by the reticuloendothelial (RE) cells within the lymph node. When a radioisotope is incorporated in such a pharmaceutical suitable for lymphatic accretion, the lymph system, particularly the draining lymph nodes, can be imaged with a suitable scintigraphic system.

However, when a disease process impacts upon these lymphatic structures, the image of the lymph nodes may be affected in such a manner that their form and appearance is different. For example, a cancer infiltrating a lymph node may replace a large enough portion of the RE tissue in the node to exclude an imaging agent, e.g., a radiocolloid, from that area of the node, resulting in a "negative" image effect. Similar results may be obtained when the lymph node structure and function is compromised by infectious agents, e.g., bacteria, fungi, parasites and viruses.

However, the use of such lymphoscintigraphic methods can present problems in diagnostic interpretation, since "absent" lymph nodes or "decreased uptake of radioactivity" are not in themselves diagnostic of neoplastic or other involvement of the lymph nodes. Moreover, there may be lymph nodes that appear normal in the lymphoscintigram, or even show increased radiocolloid uptake, when these nodes are found to have metastases upon microscopic examination. Conversely, nodes with no apparent metastatic involvement may show decreased or no radiocolloid uptake. Thus, a method with greater specificity for lymph node involvement in cancer or in infectious diseases would be of considerable diagnostic value.

Methods of localization and therapy of tumors and infectious lesions using labeled antibodies and antibody fragments which specifically bind markers produced by or associated with tumors or infectious lesions have been disclosed, inter alia, in Hansen et al., U.S. Patent 3,927,193 and Goldenberg, U.S. Patents 4,331,647, 4,348,376, 4,361,544, 4,468,457, 4,444,744, 4,460,459 and 4,460,561, and in European Patent Applications Nos. 0131627 and 0149709, the disclosures of all of which are incorporated herein in their entireties by reference. See also DeLand et al., J. Nucl. Med., 20, 1243-50 (1979).

EP-A-035265 relates to a tumour imaging method which uses a labelled antibody specific to a tumour antigen and a second, differently labelled and non-specific antibody. Thus, the tumour-specific antibody produces a positive image of a tumour which produces or is associated with the antigen and the second, nonspecific antibody will provide a general background image which can be subtracted from the positive image of the tumour. The relevant background art disclosed in EP-A-035265 relates to CEA and the use of anti-CEA antibodies to localise tumours by scintillation scanning.

WO-A-8500522 (corresponding to EP-A-0 149 709) discloses an imaging technique which uses an antibody specific to a tumour-associated marker. The purpose of the disclosed imaging technique is to enhance target specificity of antibody localisation and clearance of non-target diagnostic and therapeutic agents. This improvement is accomplished by first injecting a subject with a tumour marker-specific antibody and then, at a time after injection, injecting the subject with a second unlabelled antibody specific against the first antibody, in an amount which is sufficient to decrease the level of circulating first marker-specific antibody, and then scanning with a detector to detect and locate the site or sites of uptake of the first antibody by the lesion. The relevant background art disclosed in WO-A-8500522 relates to method of tumour localisation and therapy using labelled antibodies and antibody fragments.

These methods use radiolabeled antibodies which specifically bind to markers produced by or associated with tumors or infectious lesions, and result in a "positive" image, i.e., uptake of radioactivity attached to the antibody in the structure involved with tumor or infectious lesion and having the appropriate antibody target, thus permitting a visualization of the involved structure. Further improvements in the specificity and resolution of these methods is achieved by the use of various subtraction techniques which are also disclosed in the aforementioned references, and which enable background, non-specific radioactivity to be distinguished from specific uptake by the tumor or lesion.

Others have employed lymphoscintigraphy to study various types of cancers, using various imaging agents. Current lymphoscintigraphic methods employ Tc-99m antimony sulfide colloid (Tc-ASC) as the imaging agent of choice, although Tc-99m stannous phytate has also been reported as useful. See, e.g., Ege et al., Brit. J. Radiol., 52, 124-9-(1979); and Kaplan et al., J. Nucl. Med., 20, 933-7-(1979). Earlier, Au-198 colloid was used, as reported by, e.g., Hultborn et al., Acta Radiol., 43, 52-(1955); Turner-Warwick, Brit. J. Surg., 46, 574-(1959); Vendrell-Torne et al., J. Nucl. Med., 13, 801(1972); Robinson et al., Surg. Forum, 28, 147-(1977); Sherman et al., Am. J. Roentgenol., 64, 75-(1950); and Rosse et al., Minerva Med., 57, 1151-(1966). Intraperitoneal autologous Tc-99m-labeled erythrocytes were used in mediastinal lymphoscintigraphy to study ovarian cancer by Kaplan et al., Br. J. Radiol., 54, 126(1981). Tc-99m-labeled liposomes were used in axillary lymphoscintigraphy of breast cancer by Osborne et al., Int. J. Nucl. Med. Biol., 6, 75(1979). Tc-99m rhenium sulfide colloid was used in breast cancer lymphoscintigraphy by Gabelle et al., Nouv. Presse Med., 10, 3067(1981). The use of Tc-ASC for lymphoscintigraphic imaging of mammary and prostatic cancers, as well as for malignant melanoma, has been reported by, e.g., Ege, Sem. Nucl. Med., 13, 26(11983); Ege, J. Urol., 127, 265-9 (1982); and Sullivan et al., Am. J. Radiol., 137, 847-51(1981).

DeLand et al., Cancer Res., 40, 2997-3001 (1980), disclose a scintigraphic imaging method using anti-carcinoembryonic antigen antibodies labeled with I-131. They found that the tumor marker, carcinoembryonic antigen (CEA), was accumulated in lymph node metastases and also in some non-metastatic lymph nodes in the drainage path of proximal tumors, and was revealed by binding to labeled antibody.

Lymph nodes have been imaged by magnetic resonance imaging techniques, but not with the use of image enhancing contrast agents, and not with antibody-conjugated imaging agents.

It is important in certain clinical situations to detect the presence or absence of a particular organ, such as the ovary. Moreover, it is often necessary to determine whether an organ is anatomically correct and whether it has pathology, e.g., obstruction, infection, neoplasia and the like, by a non-invasive technique. It would be desirable to have an organ imaging method using organ-specific imaging agents that would make it possible to obtain a "positive" image of the organ, when normal,and a defect in organ visualization if pathology is present. Such a method would provide a new approach to scintigraphic and magnetic resonance imaging of organs and tissues in the body based upon their immunological specificity.

Antibody conjugates comprising organ-specific and tissue-specific antibodies and addends for scintigraphic detection or magnetic resonance image enhancement have not been used as organ imaging reagents.

A need continues to exist for lymphographic imaging methods which are more sensitive and specific for tumor and infectious lesion involvement in lymphatic structures, and for organ imaging reagents and methods with high specificity for differentiation of particular organs and tissues from surrounding structures.

OBJECTS OF THE INVENTION

One object of the present invention is to provide a method for obtaining lymphoscintigraphic images that permits higher resolution and greater specificity for tumor or infectious lesion involvement with lymphatic structures.

Another object of this invention is to provide lymphomagnetic resonance imaging methods using image enhancing agents having organ-specific and/or tumor or lesion-specific properties as well as gross image enhancing agents.

Yet another object of the invention is to provide methods for lymphoscintigraphy and magnetic resonance lymphography which permit convenient subtraction of other organs such as the liver and spleen.

A further object of this invention is to provide organ-specific methods and agents for scintigraphic and magnetic resonance imaging.

Yet a further object of the invention is to provide reagents and kits suitable for use in the lymphographic imaging methods of the invention.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the art.

SUMMARY OF THE INVENTION

These objects can be achieved by providing, in a first aspect of the invention, the use of an antibody or antibody fragment which specifically binds to a marker produced by or associated with normal cells or tissues of an organ, the antibody or antibody fragment being labeled with a radioisotope catable of external detection or with a magnetic resonance imaging enhancing agent in the preparation of a material for use in a method of organ imaging in a mammalian subject by scintigraphy or magnetic resonance imaging, comprising the steps of:

(a) parenterally injecting a mammalian subject at a locus and by a route providing access to said organ with the said labeled antibody or antibody

fragment, the amount of the labeled antibody or antibody fragment being sufficient to permit a scintigraphic image or an enhanced magnetic resonance image of the organ to be obtained; and

(b) obtaining a positive scintigraphic image or a positive enhanced magnetic resonance image of the organ at a time after injection of said antibody or antibody fragment sufficient for the antibody or antibody fragment to accrete in the organ and specifically bind to the marker; wherein a tumor or infectious lesion of said organ will be revealed as a negative scintigraphic or enhanced magnetic resonance image.

Preferred embodiments of this aspect of the invention are:

wherein said antibody/fragment is labeled with a radioisotope;

wherein said antibody/fragment is labeled with a magnetic resonance image enhancing agent;

wherein said radioisotope emits gamma radiation in the range of 50-500 keV;

wherein said antibody/fragment is labeled with a radioisotope capable of simultaneous independent detection in the presence of Tc-99m; wherein the antibody/fragment label is I-131 or In-111; and/or

wherein said antibody/fragment is labeled with a magnetic resonance image enhancing agent comprising at least one of Gd(III), Eu(III), Dy(III), Pr-(III), Pa(IV), Mn(II), Cr(III), Co(III), Fe(III), Cu(II), Ni-(II), Ti(III) or V(IV) ions or a nitroxide radical;

In a preferred embodiment, the imaged organ is a lymphatic structure, and in this case, the antibody or antibody fragment binds specifically to normal lymphatic cells or tissues.

In another preferred, embodiment, the method further includes the steps of :

(c) simultaneously or at an earlier or later time, parenterally injecting the subject, at the same or different locus and by the same or different route, with a further antibody or antibody fragment which specifically binds a marker produced by or associated with a tumor or infectious lesion, said further antibody or antibody fragment being labeled with a radioisotope capable of external detection or with a magnetic resonance image enhancing agent, the amount of the labeled further antibody or antibody fragment being sufficient to permit a positive scintigraphic image or a positive enhanced magnetic resonance image of the tumor or infectious lesion to be obtained;

(d) obtaining a positive scintigraphic image or a positive enhanced magnetic resonance image of the tumor or infectious lesion, at a time after injection of the further labeled antibody or antibody fragment sufficient for the further antibody or antibody fragment to become specifically bound to the marker produced by or associated with the tumor or infectious lesion; and

(e) subtracting the image obtained in step (b) of the method set forth above, which is a gross image including a positive image of normal tissues and cells of the organ and negative image of any tumor or infectious lesion or accretion of a localised product thereof, from the image obtained in step (d) above, which is a positive image of the tumor or infectious lesion or accretion of a localised product thereof, to produce a refined positive image of the tumor, infectious lesion or accretion of a localised product thereof.

In a further embodiment, said antibody/fragment comprising said gross imaging agent also specifically binds to at least one of normal liver and spleen tissue; wherein at least a portion of said gross imaging agent is injected by a systemic route; and wherein in step (b), said image includes an image of at least one of the liver and the spleen, and in step (e), said image of at least one of the liver and the spleen is also subtracted from the image obtained in step (d).

Preferably, said systemic route of injection is intravenous, intraarterial, intramuscular or subcutaneous.

In yet another preferred embodiment, wherein said gross imaging agent specifically binds to a lymphocyte marker, said marker is a T-cell marker or a B-cell marker.

Still further improvements can be obtained by using antibodies to normal lymph node structures and/or tissues as the non-specific imaging agent, and subtracting the resultant image from the positive image obtained using antibodies which specifically bind to tumors or infectious lesions.

The invention further provides a scintigraphic imaging kit for imaging a mammalian lymphatic structure, comprising:

(a) at least one gross lymphatic imaging agent, which comprises an antibody or antibody fragment which specifically binds to normal lymphatic cells or tissues, said antibody or antibody fragment being labeled with a radioisotope label or a magnetic resonance image enhancing agent;

(b) at least one antibody or antibody fragment which specifically binds a marker produced by or associated with a tumour or infectious lesion, said antibody or antibody fragment being labelled with a radioisotope or a magnetic resonance image enhancing agent;

(c) a sterile, pharmaceutically acceptable injectable vehicle.

DETAILED DISCUSSION

In one methodological aspect, the present invention combines two approaches hitherto employed separately, in a way which has not been suggested in the earlier work on either technique. The work of DeLand, in collaboration with the present inventor, was related to localization of radiolabeled antibodies in tumors or accreted antigen foci of the lymphatics. The work of others was related to lymphoscintigraphic imaging of lymphatics with gross imaging agents. The present invention relates to the hitherto unsuggested correlation and computer processing of these two images to refine the positive image of a tumor or other pathological lesion, or accreted antigen focus, revealed by specific antigen-antibody binding.

The lymphographic method of the invention can be practiced either with scintigraphic or magnetic resonance imaging agents. A combination of these imaging agents can also be used, although this requires more complex instrumentation and data processing and may therefore be impractical in most cases. The subtraction of images can be readily achieved using conventional software. The imaging methods of DeLand et al., Cancer Res., 40, 3046(1980), are illustrative of the computerized subtraction methods known in the art.

Major areas of interest for lymphography include regional spread of neoplastic and infectious lesions of the breast, colon and rectum, prostate, ovary and testes. Major lymph nodes involved in these various lesions include axillary and internal mammary nodes in the chest, and the pararectal, anterior pelvic (obturator), internal iliac (hypogastric), presacral, external and common iliac, and para-aortic nodes. Thus, applications where lymphographic imaging would be useful include, but are not limited to, pathological lesions affecting the major organs of the chest, abdomen and pelvis, as well as the skin, from which the regional and, subsequently, more distant lymphatics can be involved.

Scintigraphic imaging according to the method of the invention is effected by obtaining a scintigram of the lymphatic structure of interest, using as an imaging agent a radiolabeled antibody which specifically binds to a marker produced by or associated with a tumor or infectious lesion located in the structure or at a locus proximal to the structure and draining into the structure, such that the antigen/marker accretes in discrete foci therein; also obtaining a scintigram of the structure using a gross imaging agent which is a radiolabeled material which accretes in the structure but which does not specifically bind to the tumor or lesion, or to an accreted antibody focus; and subtracting the latter image from the former to produce a refined positive image of the site or sites of localization of the labeled specific antibody within the structure.

The "gross" labeled imaging agent is a new type of gross imaging agent developed especially for this invention, namely an antibody, preferably a radiolabeled antibody, which specifically binds to normal lymphatic tissues or cells, but not to tumors or lesions located therein or proximal to and draining into the structure, so that it is also diffusely distributed in the lymph nodes and reveals the internal structure thereof.

Radiolabeled antibodies to markers characteristic of lymphatic tissue are a new kind of gross imaging agent which are especially useful in the method of the present invention. They are an example of an immunologio, organ-specific imaging agent which can be used to ascertain the location and shape of a specific organ and reveal possible abnormalities therein. Such agents are useful for imaging organs other than lymphatics, e.g., liver, spleen, pancreas, and the like, and many antibodies which specifically bind to tissues of these organs are known and/or under current investigation and development.

Organ-associated and organ-specific antibodies can be developed by immunizing a suitable animal host with certain mammalian tumors or normal organ/tissue extracts and/or cells. It is well known that use of tumors as immunogens can result in antibodies which not only react with neoplasia but also with normal tissue components which sometimes show an organ-restricted nature. Histogenetic and functional differences between various tissues and organs of the body of course suggest that distinct antigens are present and identifiable. A body of scientific literature already exists which claims the identification of organ-specific antigens, either using classical immunization approaches or by immunizing with specific tumors, and this is reviewed by Goldenberg et al., Cancer Res., 36, 3455(1976), showing that such antigens are known and available.

Similar organ- and tissue-associated and specific antigens are identifiable by hybridoma methods which produce monoclonal antibodies. One recent development is the production of human hybridoma monoclonal antibodies by securing lymphocytes or plasma cells from patients showing certain organ-restricted autoimmune diseases, e.g., thyroiditis, gastritis, ulcerative colitis, myositis, and the like. These antibody-producing cells are then fused in vitro with human or murine myeloma cells and hybridomas of appropriate anti-organ and anti-tissue antibody formation are produced and propagated, using well known methods. Also, patients with specific tumor types can be used as a source of such lymphocytes or plasma cells, or such patients can be further immunized with such tumor

cells for stimulating the production of anti-organ and anti-tissue antibodies. The lymphatic tissue removed is then used for fusion with suitable myeloma cells, by procedures which are by now well known and conventional in the art.

Organ-associated and organ-specific antigens can be isolated for immunization of another species, e.g., sub-human primates, rodents, rabbits, goats, etc., by a number of methods known in the art, such as isolation of cell membranes or disruption of the cells, e.g., by centrifugation, sonication, etc., to obtain intracellular antigens. It is preferable, for these purposes, to use intracellular as opposed to surface and extracellular antigens. In this manner, organ-associated and organ-specific antigens can be obtained from a large number of tissues and organs of the body, including brain, thyroid, parathyroid, larynx, salivary glands, esophagus, bronchus and lungs, heart, liver, pancreas, stomach and intestines, kidney, adrenal gland, ovary, testis, uterus, prostate, etc. Of further interest is the differentiation of different tissue and cellular components within an organ, such as tubular and glomerular kidney, different regions and cell types of the brain, endocrine and exocrine pancreas, etc., especially by the identification of antigens and antigen epitopes restricted to the individual cell and tissue types in question, as accomplished with polyclonal and/or hybridoma-monoclonal antibody-production methods known in the art.

Examples of antibodies which specifically bind to lymphatic cells and/or tissues, and which are useful as gross imaging agents when labeled with a radioisotope or magnetic resonance image enhancing agent, include the T101 murine monoclonal anti-T-cell antibody reported by Royston et al., Blood, 54(Suppl. 1), 106a(1979); and the T200 anti-lymphoreticular cell monoclonal antibody whose specificity was reported by Hsu et al., Am. J. Pathol., 114, 387 (1984). Other antibodies to T-cells and B-cells, which can also be used for such agents, include, e.g., the B1, B2 and BA1 anti-B-cell monoclonal antibodies reported in Hsu et al., Am. J. Clin. Pathol., 80, 415 (1983), and in Hsu et al., Am. J. Pathol., 114, 387 (1984); the OKT10, A1G3, HLA-DR and Leu 10 monoclonals reported in Hsu et al., Ibid.; and anti-lymphocyte monoclonals reported by Foon et al., Blood, 60, 1 (1982), LeBien et al., J. Immunol., 125, 2208(1980), and Beverley et al., Eur. J. Immunol., 11, 329-(1981).

The antibody may be whole IgG, IgA, IgD, IgE, IgM or a fragment such as, e.g., F(ab')2, F(ab)2, Fab', Fab or the like, including isotypes and subtypes thereof. It can be a polyclonal antibody, preferably an affinity-purified antibody from a human or an appropriate animal, e.g., a goat, rabbit, mouse or the like, or a monoclonal antibody prepared by conventional techniques, e.g., a murine antibody derived from a hybridoma produced by fusion of lymph or spleen cells from a mouse immunized against a lymphatic system antigen with myeloma cells from an appropriate immortal cell line.

It should be noted that mixtures of antibodies, isotypes, and immunoglobulin classes, including fragments, can be used, as can hybrid antibodies and/or antibody fragments. In particular, hybrids having both T101 and T200 specificities, or hybrids having anti-T-cell and anti-B-cell specificities, may be particularly useful as gross lymphatic imaging agents, both for scintigraphy and for magnetic resonance lymphography, depending upon the label or enhancing moiety conjugated thereto. Hybrid antibody fragments with dual specificities can be prepared analogously to the anti-tumor marker hybrids disclosed in U.S. Patent 4,361,544. Other techniques for preparing hybrid antibodies are disclosed in, e.g., U.S. Patents 4,474,893 and 4,479,895, and in Milstein et al., Immunol. Today, 5, 299(1984).

The antibody/fragment used for the specific imaging agent can be any of the antibodies which bind to tumor-specific and/or tumor/associated markers such as those disclosed in the herein referenced U.S. Patents and Patent Applications, including hybrid antibodies and/or fragments, as well as others which are known to the ordinary skilled artisan in this field, e.g., antibodies which bind to human T-cell lymphoma viruses (HTLV), and those which are yet to be discovered. Also useful are antibodies to markers produced by or associated with infectious lesions of the lymphatic system, or lesions located proximal to and draining into lymph nodes. Lymphotropic micro-organisms include bacteria. viruses, parasites, and the like which show a predilection for sojourn in and involvement of lymphatic structures in the body. Among the viruses, the HTLV family have a predilection for T-lymphocytes, and are involved in leukemias, lymphomas, and AIDS. The HTLV form considered etiologic for AIDS is HTLV-III. Cytomegalovirus, EB herpes virus, and the like, also show some predilection for lymphatic structures although the site of primary infection can be other tissues, with subsequent involvement of lymphatic tissues. However, virtually all pathogenic microorganisms can demonstrate involvement of lymphatic tissues during passage and infection in the body.

Examples of antibodies to infectious organisms and/or antigens produced by or accreted by or in the vicinity of infectious lesions include, e.g., antibodies against variola virus, yellow fever virus, arboviruses, herpes viruses, myxoviruses, enteroviruses, rabies virus, hepatitis A and B viruses,

Chlamydia psittaci, Rickettsia prowazeki and other rickettsia, lymphccytic choriomeningitis virus, Neisseria meningitidis, Neisseria gonorrhoeae, Corynebacterium diphtheriae, Clostridium tetani, Bacillus anthracis, Yersinia pestis, Vibrio cholerae. salmonella and shigella bacterial species, staphylococci species, Reponema pallidum, leptospiral species, Mycobacterium leprae, Mycobacterium tuberculosis, Histoplasma capsulatum, Coccidioides immitis, various streptococci, Plasmodium falciparum and other plasmodia, Toxoplasma gondii, Leishmania donovani, various trypanosomes, Entameba histolytica, Giardia lambia, Trichinella spiralis, Strongyloides stercoralis, Antiostrongylus cantonensis, Wucheria bancrofti, Schistosoma mansoni and other schistosomal helminths, Paragonimus westermani, echinococcal species, and the like. Listings of representative disease-causing infectious organisms to which antibodies can be developed for use in this invention are contained in the second and subsequent editions of Davis et al, "Microbiology" (Harper & Row, New York, 1973 and later), and are well known to the ordinary skilled art worker.

Again, the antibody may be whole IgG, IgA, IgD, IgE, IgM or a fragment such as, e.g., F(ab')2, F(ab)2, Fab', Fab or the like, including isotypes and subtypes thereof. It can be a polyclonal or a monoclonal antibody/fragment, a mixture of antibodies/fragments or a hybrid. Here, where the image is produced as a result of specific antibody-antigen binding rather than non-specific uptake by the RES, it may be especially advantageous to use antibody fragments which do not have the Fc portion.

The radiolabel for both types of scintigraphic imaging agents is preferably an isotope with an energy in the range of 50-500 Kev. Where more than one isotope is used for simultaneous subtraction, the two labels should be of sufficiently different energies to be separately detectable with a gamma camera having a collimator with the appropriate characteristics.

The type of gross imaging agent disclosed hereinabove, i.e., an antibody to a marker associated with lymphatic tissue, can be prepared by known methods, if existing antibodies are considered unsuitable or if different or more discriminating specificities are desired. Generally, whole lymph cells, tissue samples and/or cell or tissue fractions, membranes, antigen extracts or purified surface antigens are used to challenge the immune system of a suitable animal, e.g., a mouse, rabbit, hamster, goat or the like, the antigen being rendered immunogenic by aggregation if necessary and/or by coadministration with a suitable conventional adjuvant. Hyperimmune antiserum can be isolated and polyclonal antibodies prepared by conventional procedures. Alternatively, spleen cells can be fused with immortal myeloma cells to form hybridoma cells producing monoclonal antibodies, by what are now conventional procedures. See, e.g., the procedures in the above-referenced European Patent Application No. 0131627 for illustrative techniques. Hybridomas using animal, e.g., mouse, or numan myeloma cell lines and animal or human spleen or lymph cells are all known in the art, and can be bade and used for the present method. See, for example, Glassy et al., Human Monoclonal Antibodies to Human Cancers", in "Monoclonal Antibodies and Cancer", Boss et al., Eds., 163-170 (Academic Press, 1983). The specific antisera or monoclonals are screened for specificity by methods used to screen the anti-lymphocyte clones in the references cited hereinabove, which methods are also conventional by now in this art.

In an alternative embodiment of this approach, the gross agent can be a labeled antibody to a marker associated with a lymphatic structure, e.g., lymphatic tissues or lymphocytes wherein the antibody also specifically binds to a marker produced by or associated with liver and/or spleen tissues or components. Among the anti-lymphatic clones disclosed hereinabove, at least the anti-T101 antibody is also cross-reactive with spleen. Antibodies which are cross-reactive with both lymphatic tissue/cells and liver and/or spleen cells/tissue can also be prepared by well-known hybrid antibody production techniques, such as those disclosed in the above-referenced U.S. 4,331,647, 4,474,893 and 4,479,895. These would combine anti-lymph tissue antibodies with antibodies which specifically bind to liver and/or spleen.

Such antibodies can be produced using liver cells isolated from normal liver tissue obtained at autopsy. For example, mice can be immunized with such tissues for a period necessary to evoke anti-liver antibodies. The spleens of these mice are removed and then fused, by standard methods, with a murine myeloma cell line suitable for hybridoma production. Using methods already standard in the art, monoclonal antibody-producing hybridomas are selected and propagated, and those with liver-restricted or liver-associated antibody production are cloned and expanded as a source of liver organ antibodies.

Similar approaches can be used with other normal human tissues for the production of antibodies that are organ-associated or tissue-specific. Absolute tissue specificity is not required since significant quantitative differences ordinarily suffice for operational specificity for imaging purposes.

It will also be appreciated that the anti-liver antibodies can be used as a liver background subtraction agent when visualizing tumors in the liver.

These tumors can be of non-liver origin or of liver origin. Even if a tumor of liver origin has the liver organ-associated antigen, subtraction of the latter can be accomplished without missing the tumor if another liver cancer-associated antigen is used as the target for the specific anti-liver cancer antibody. For example, antibody against alpha-fetoprotein (AFP) can be used in combination with an antibody against normal liver organ antigen, thus refining the image of areas containing AFP in the liver.

The antibodies can be radiolabeled by a variety of methods known in the art. Many of these methods are disclosed in the above-referenced U.S. Patents and Patent Applications, and include direct radioiodination, chelate conjugation, direct metallation, and the like. See also, Rayudu, op. cit.; and Childs et al., J. Nuc. Med., 26, 293(1985). Any conventional method of radiolabeling which is suitable for labeling isotopes for in vivo use will be generally suitable for labeling imaging agents according to the present invention.

The gross imaging agent will normally be administered at a site and by means that insure that it is mobilized and taken up into the lymphatic circulation. This will vary with the system to be imaged. Multiple injection sites may be preferable in order to permit proper drainage to the regional lymph nodes under investigation. In some cases, injections around the circumference of a tumor or biopsy site is desired. In other cases, injection into a particular sheath or fossa is preferred. Injection into the webs of the fingers or toes is a common mode used to study peripheral lymphatics.

In patients with breast carcinoma, a unilateral injection is made in the subcostal site ipsilateral to the tumor, and then repeated later on the contralateral side to observe cross drainage between the ipsilateral and contralateral nodes. Imaging is effected at appropriate times after each injection and injection of the specific imaging agent is coordinated with the injections of the gross imaging agent to permit optimal visualization of the positive and negative images.

Images of axillary, subclavian and supraclavicular nodes may be obtained by injecting the imaging reagents into the medial surface of the upper arms (ipsilateral and contralateral) of patients with breast cancer.

In certain cases, such as testicular or prostatic cancer or some cases of rectal carcinoma, intratumoral or peritumoral injection of imaging agents can be effective.

The cross-reactive agent is preferably injected by a systemic route, e.g., intravenously, intraarterially, intramuscularly or subcutaneously, or by a combination of systemic and intralymphatic routes insuring its accretion in both the lymphatic structure of interest and the liver and/or spleen. This technique permits subtraction of the liver and/or spleen which can further refine the image of the desired lymphatic structure. Another advantage of this approach is its utility in reducing repositioning errors in sequential imaging wherein a patient is imaged in multiple sessions. The organ image can be used to correlate and superimpose temporally discrete images by computer matching of the organ image from the separate sessions.

Volumes of labeled antibody gross imaging agent, normally in sterile phosphate-buffered saline (PBS) solution or sterile mineral oil suspension, will normally vary somewhat depending upon the site, the concentration and activity of the preparation, and the number of injections.

Activity of the gross agent will normally be in the range of about 0.1-2.5, preferably about 0.25-1.5, mCi per injection for a Tc-99m-labeled agent. It will be appreciated that the activity will vary for other radioisotopes, depending upon their half-lives, their imaging characteristics, i.e., energy ranges, emission intensities, scatter and the like, the stability of the labeled agent, especially antibody conjugates, their rate of transport to the lymph nodes, their distribution and clearance, and the time at which imaging is to be done. Adjustment of these parameters will be conventional for the ordinary skilled clinician.

Imaging is normally effected up to about 6 hours, more preferably at about 2 - 4 hours after injection of the gross imaging agent, to obtain the "negative" image of the lymphatic structure. Imaging of the localized specific imaging agent is normally effected at about 12 - 48 hours, preferably at least about 24 hrs post-injection, in order for the non-specifically bound antibody to clear the node. If too much of the specific agent enters the circulation, conventional subtraction agents, e.g., 99m-pertechnetate and Tc-99m-HSA can be used to normalize. Alternatively, second antibody, e.g., rabbit or goat anti-mouse IgG, can be injected i.v. to enhance clearance of the specific antibody, as disclosed in EP-A-0149709.

Timing of the injections of gross and specific imaging agents will depend upon the types of agents used and the drainage patterns to the nodes of interest. Normally, it will take the specific agent a longer time to localize, and for the non-localized agent to clear the nodes, than the time required before imaging can be effected with the gross imaging agent. Thus, if it is desired to image both agents at about the same time, the specific imaging agent may need to be injected well before the gross agent. DeLand et al., 1980, loc. cit., reported imaging at between about 6 and 48 hours post-injection for breast cancer cases, where I-131-labeled anti-CEA antibody was injected in the webs of the fingers and feet.

It is generally preferred to effect imaging of both the gross and specific agents at the same time, using separately detectable radionuclides. This avoids the errors associated with repositioning the patient and/or realigning the images by computer. Consequently, the choice of label for the gross and specific imaging agents and the activities thereof will take into consideration the time intervals for imaging. The specific antibody imaging agent normally will have a label with at least as long a half-life as the gross agent. In the earlier example hereinabove, the antibody is labeled with I-131, and the gross agent has a Tc-99m label. The antibody could be labeled with In-111 and the gross agent with Ga-67, both of which have about the same half-life of about 2.5 days. Other pairs of compatible radionuclides for use in labeling the specific and gross imaging agents are disclosed in, e.g., the above-referenced U.S. Patent 4,444,744.

The scintigram is normally taken by a gamma imaging camera having one or more windows for detection of energies in the 50-500 keV range. Use of radioisotopes with high enough energy beta or positron emissions would entail use of imaging cameras with the appropriate detectors, all of which are conventional in the art.

The scintigraphic data are stored in a computer for later processing. Subtraction of the "negative" image obtained with the gross imaging agent sharpens and refines the "positive" image obtained with the specific, localized labeled antibody . Subtraction is effected by the method of DeLand et al., op. cit., or variants thereof, according to well-known techniques of data processing, normally involving pixel-by-pixel subtraction of normalized values of counts for each channel of the detector, optionally with correction for the counting efficiency of each channel for the radionuclide label detected therein, and conversion of the subtracted values to an output signal to a monochrome or color screen. Where cross-reactive antibodies are used as the gross imaging agent, computer subtraction of the image of the cross-reactive organ is also effected to further resolve the positive image of the localized antibody site or sites.

If no tumor or lesion is present in the structure, but marker accretes there by drainage from a proximal tumor or infection, the marker can accrete in discrete foci within the lymph nodes in the drainage path. This can be visualized using the present method, since the gross imaging agent will still enable subtraction of areas of only diffuse accretion. The diagnostic significance of such fool of antigen accretion may be difficult to evaluate, and to distinguish from small metastases, but this problem is common to earlier methods and must be resolved by correlation of imaging data with other diagnostic results. It will be recognized that use of only gross imaging agents fails to reveal such antigen localization, i.e., foci of antigen accretion, which often suggest eventual invasion of tumor cells and also reveal tumor drainage pathways.

Another important application of the organ- or tissue-specific or organ- or tissue-associated antibodies disclosed hereinabove is for normal organ scintigraphy and mri. In this case, a suitably radiolabeled antibody/fragment or an antibody/fragment bearing a mr image enhancing agent is administered with the intention of obtaining a "positive" image of the organ, when normal, and a defect in organ visualization if pathology is present. This provides a new approach to organ and tissue-specific nuclear and magnetic resonance imaging of organs and tissues in the body, based upon their immunological specificity.

It will be understood that the invention is not limited to use of known antibodies or markers, but can be practiced with antibodies to any marker produced by or associated with a tumor or other pathological lesion.

Magnetic resonance imaging (mri) is effected in an analogous manner to scintigraphic imaging except that the imaging agents will contain magnetic resonance (mr) enhancing species rather than radioisotopes. It will be appreciated that the magnetic resonance phenomenon operates on a different principle from scintigraphy. Normally, the signal generated is correlated with the relaxation times of the magnetic moments of protons in the nuclei of the hydrogen atoms of water molecules in the region to be imaged. The magnetic resonance image enhancing agent acts by increasing the rate of relaxation, thereby increasing the contrast between water molecules in the region where the imaging agent accretes and water molecules elsewhere in the body. However, the effect of the agent is to decrease both T_1 and T_2, the former resulting in greater contrast while the latter results in lesser contrast. Accordingly, the phenomenon is concentration-dependent, and there is normally an optimum concentration of a paramagnetic species for maximum efficacy. This optimal concentration will vary with the particular agent used, the locus of imaging, the mode of imaging, i.e., spin-echo, saturation-recovery, inversion-recovery and/or various other strongly T_1-dependent or T_2-dependent imaging techniques, and the composition of the medium in which the agent is dissolved or suspended. These factors, and their relative importance are known in the art. See, e.g., Pykett, Scientific American, 246, 78(1982); Runge et al., Am. J. Radiol., 141, 1209(1983).

Again, the gross agent is a labeled antibody to a normal component of lymphatic structures, labeled with a paramagnetic ion or radical which can significantly alter the relaxation time of protons in

water molecules in its vicinity. It is also possible to use an agent containing a high concentration of atoms of an element other than hydrogen, having a strong nuclear magnetic moment which is detectable by an nmr detector, e.g., Fluorine-19 and the like, and which can also be accreted in a lymphatic structure in an amount sufficient for efficient nmr detection.

Examples of agents useful for mri of lymphatic systems include Gd(III), Eu(III), Dy(III), Pr(III), Pa(IV), Mn(II), Cr(III), Co(III), Fe(III), Cu(II), Ni(II), Ti(III) and V(IV) ions other strongly paramagnetic ions, or radicals, e.g., nitroxides, as antibody conjugates bearing paramagnetic ion chelates or radical addends. The latter will include paramagnetic conjugates with antibodies to lymphatic structures or lymphocytes, for use as gross imaging agents, as well as conjugates with antibodies to tumor or lesion markers for use as specific imaging agents.

The specific imaging agent can use the same image enhancing agent, with mri effected at different times from the gross imaging, or a label which is separately and independently detectable with an nmr imaging camera, in the presence of the agent used for gross imaging. Examples of the latter strategy include, e.g., use of antibody conjugates with heavy loadings of Gd(III) or Mn(II) chelates as the specific imaging agent, where the gross imaging agent is a colloid containing a high concentration of fluorine atoms or atoms of another suitable element having a strong nuclear magnetic moment, whose nuclear magnetic resonance frequency occurs at a widely different value from that of the hydrogen nucleus.

The mr image enhancing agent must be present in sufficient amounts to enable detection by an external camera, using magnetic field strengths which are reasonably attainable and compatible with patient safety and instrumental design. The requirements for such agents are well known in the art for those agents which have their effect upon water molecules in the medium, and are disclosed, inter alia, in Pykett, op. cit., and Runge et al., op. cit.

Preparation of antibodies conjugated to a magnetic resonance image enhancing agent can be effected by a variety of methods. In order to load an antibody molecule with a large number of paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, e.g., ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and the like groups known to be useful for this purpose. The chelate is normally linked to the antibody by a group which enables formation of a bond to the antibody with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other, more unusual, methods and reagents for conjugating chelates to antibodies are disclosed in copending European Patent Application No. 86304352.7 (inventor: Hawthorne), entitled "Antibody Conjugates", filed June 7, 1985.

The mr scans are stored in a computer and the image subtraction is effected analogously to the scintigraphic data.

Reagents for use in the method of the invention include radiolabeled antibodies/fragments which specifically bind to markers produced by or associated with tumors and infectious lesions, radiolabeled antibodies/fragments which specifically bind to lymphatic structural components, including tissues and lymphocytes, radiolabeled antibodies/fragments which specifically bind to normal organ tissues, and the analogous imaging agents labeled with mr image enhancers, as disclosed hereinabove. These will be packaged separately or together, depending upon whether they are to be injected simultaneously or separately, or whether or not they are labeled at the site of administration or at a remote location.

The reagents are conveniently provided in kit form, adapted for use in the method of the invention. Kits will normally contain separate sealed sterile vials of injectable solutions of labeled reagents, or lyophilyzed antibodies/fragments or antibody/fragment conjugates and vials of suitable conventional injection vehicles with which they will be mixed just prior to administration.

Kits may also include reagents for labeling antibodies, e.g., Chloramine-T (for I-131 or I-123 labeling), $SnCl_2$ (for Tc-99m labeling using pertechnetate from a commercial generator), short columns for sizing and/or purification of reagents, and other conventional accessory materials.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the following examples, all temperatures are set forth uncorrected in degrees Celsius; unless otherwise indicated, all parts and percentages are by weight.

EXAMPLE 1

Preparation of lymphoscintigraphic reagents.

a) Ga-67-labeled T101 anti-lymphocyte monoclonal antibody

A sample of T101 anti-lymphocyte murine monoclonal antibody, as reported by Royston et al., loc. cit., is labeled with Ga-67 by the method of Hnatowich et al., Science, 220, 613(1983), to form the conjugate with a diethylenetriaminepentaacetate (DTPA) gallium(III) chelate, containing an average of 4 Ga atoms per antibody molecule, and retaining at least 70% of its initial immunoreactivity. A solution of the antibody in PBS, pH 7.4, is added to a 50-fold molar excess of solid DTPA dianhydride, and agitated for 5 minutes. Free DTPA is removed by gel filtration on Sephadex G 50. About 1 mCi Ga-67 citrate is added per mg of antibody-DTPA conjugate, and incubated for 20 minutes, and unbound Ga-67 is then removed, e.g., by gel filtration on Sephadex G 50. The resultant Ga-67-DTPA-T101 has a specific activity of about 0.5 - 1.5 mCi/mg.

b) I-131-labeled T101 anti-lymphocyte monoclonal antibody

A sample of the T101 monoclonal antibody used in Example 1 is labeled with I-131 according to the procedure of Example 1(f) of U.S. Patent 4,348,376, using Chloramine-T and replacing the anti-CEA antibody used in the referenced procedure with an equal weight of T101 antibody, but reducing the amounts of reagents to lower the iodine content. The resultant I-131-T101 has an average of 1 atom of iodine per antibody molecule and a specific activity of about 12 mCi/mg.

c) In-111-labeled anti-HTLV-1 monoclonal antibody

A sample of murine monoclonal anti-HTLV-1 antibody is labeled with In-111 according to the procedure of part (a) hereof, except that In-111 oxinate is used instead of Ga-67 citrate, to form the conjugate with a DTPA indium(III) chelate, containing an average of 3 In atoms per antibody molecule, and retaining at least 70% of its initial immunoreactivity. The resultant In-111-DTPA-anti-HTLV-1 has a specific activity of about 0.5 - 1.5 mCi/mg.

d) In-111-labeled anti-prostatic acid phosphatase F-(ab')$_2$

A sample of anti-prostatic acid phosphatase (PAP) F(ab')$_2$, prepared by the method disclosed in U.S. 4,331,647, and described in Goldenberg et al., J. Am. Med Assn., 250, 630(1983), is labeled with In-111, using the procedure of part (c) hereof. The resultant In-111-DTPA-chelate conjugate contains an average of 3 In atoms per antibody fragment, and retains at least 60% of its initial immunoreactivity. It has a specific activity of about 2 mCi/mg.

e) I-123-labeled anti-CEA monoclonal antibody

A sample of the NP-2 monoclonal antibody which specifically binds to carcinoembryonic antigen (CEA), disclosed in EP-A-0131627 is labeled with I-123 according to the procedure of Example 1(f) of U.S. Patent 4,348,376, using Chloramine-T and replacing the I-131 used in the referenced procedure with an equal weight of I-123, but reducing the amounts of reagents to lower the iodine content. The resultant I-123-anti-CEA IgG has an average of 1 atom of iodine per antibody molecule and a specific activity of about 12 mCi/mg.

EXAMPLE 2

Preparation of injectable lymphoscintigraphy compositions

Sterile, pyrogen-free solutions are prepared as shown.

a) A sterile solution containing, per ml:
   1) 10 mg Human Serum Albumin (HSA) (1%, USP, Parke-Davis)
   2) 0.01 M phosphate buffer, pH 7.5 (Bioware)
   3) 0.9% NaCl
   4) 1.5 mg Ga-67-DTPA-T101 antibody prepared according to Example 1a.

b) A sterile solution according to Example 2a, except that 250 ug of the I-131-labeled antibody according to Example 1b is present instead of the Ga-labeled antibody.

c) A sterile solution according to Example 2a, except that 1.5 mg of the In-111-labeled antibody according to Example 1c is present instead of the Ga-labeled antibody.

d) A sterile solution according to Example 2a, except that 1.5 mg of the In-111-labeled antibody according to Example 1d is present instead of the Ga-labeled antibody.

e) A sterile solution according to Example 2a, except that 250 ug of the I-123-labeled antibody according to Example 1e is present instead of the Ga-labeled antibody.

EXAMPLE 3

Preparation of Reagents for NMR Lymphography

a) Preparation of Gd-labeled anti-CEA

A sample of murine monoclonal antibody to carcinembryonic antigen (CEA), prepared according to Example 2 of U.S. Patent 4, 348,376 or according to Examples 6 and 7 of EP-A-0131627 is labeled with a p-isothiocyanatobenzoyl-capped oligothiourea containing 320 Gd(III)- DTPA chelate groups prepared according to Example 11 of copending European Application No. 86304352.7, to put an average of 5 oligothiourea chains on the antibody, without loss of more than 30% immunoreactivity and without significant aggregation of the antibody conjugate. The resultant conjugate carries an average of 320 gadolinium ions thereon. The reaction is effected in 0.1 M aqueous $Na_2CO_3/NaHCO_3$ buffer, at pH 8.5, at room temperature, with at least a 50-fold excess of the polymer, and an antibody concentration of about 10 mg/ml.

The conjugate is purified by gel filtration on a column of allyl dextran cross-linked with N,N'-methylene bisacrylamide, e.g., Sephacryl S-200 - (Pharmacia Fine Chemicals, Piscatoway, N.J.).

b) Preparation of Gd(III)-labeled T101 monoclonal antibody

A sample of T101 murine monoclonal antibody is labeled with about 320 Gd ions by the procedure of part (a) hereof, except that the antibody is the T101 antibody instead of monoclonal anti-CEA IgG. The resultant conjugate is isolated by an analogous procedure to the foregoing part of this Example.

EXAMPLE 4

Preparation of injectable mri compositions

Sterile, pyrogen-free solutions are prepared as shown.

a) A sterile solution containing, per ml:
   1) 10 mg Human Serum Albumin (HSA) (1%, USP, Parke-Davis)
   2) 0.01 M phosphate buffer, pH 7.5 (Bioware)
   3) 0.9% NaCl
   4) 1.5 mg Gd-labeled anti-CEA IgG prepared according to Example 3a.

b) A sterile solution according to Example 4a, except that 1.5 mg of the Gd-labeled T101 according to Example 4b is present instead of the Gd-labeled anti-CEA.

EXAMPLE 5

Lymphoscintigraphy

A patient with carcinoma of her right breast receives injections of approximately 0.25 mCi 131-I-labeled T101 monoclonal antibody, according to Example 2(b), subcutaneously in the web of the fingers of both hands (totalling 0.7 to 1.5 mCi 131-I). The patient also receives, in the same injections, 1.5 mCi 123-I-labeled monoclonal anti-CEA antibody NP-2, according to Example 2(e), (totalling 4.2 to 9.0 mCi 123-I). Before administration of the labeled antibodies, the patient is skin-tested for allergic reaction to mouse IgG, and also receives Lugol's iodine to minimize radioiodine concentration by the thyroid gland. Immediately following the subcutaneous injection, the areas are massaged for several minutes, and the patient is asked to exercise her fingers.

By means of a gamma camera, images are obtained at frequent intervals, starting at 2 hrs. after injection and ending at 36 hrs. The data are stored in a laboratory computer and the images generated on a color display system. The 131-I images are then subtracted, pixel-by-pixel, from the 123-I images by computer processing. The metastatic foci in the ipsilateral axillary lymph node appear as early as 4 hrs., and more clearly at 6 hrs., after injection as a discrete focus of increased radioactivity, while the contralateral axillary nodes are negative. Two weeks later, the same procedure is repeated and the results confirmed, except that this time, 111-In is used to label the T101 monoclonal antibody.

Another study with 99m-Tc sulfide colloid injected simultaneously with 111-In-labeled anti-CEA monoclonal antibody, effected as above but with the appropriate doses for these radionuclides and agents, is repeated a month later and the right axillary lymph node involvement is seen again.

EXAMPLE 6

Lymphoscintigraphy

A 24 year old male presents with left side inguinal node enlargement and has a constellation of symptoms and history suggestive of AIDS. 67-Ga-labeled T101 monoclonal antibody, according to Example 2(a), is injected in divided doses into the web of the toes of both feet, with a total dose of 4 mCi. At the same time and in the same injections, an equal dose of 111-In-labeled monoclonal antibody against HTLV-1, according to Example 2(c), is injected. Beginning at 2 hrs post-injection and continuing at intervals of 2 hrs up to a total of 6 hrs, and then again at 24 hrs., the

patient's inguinal region and pelvis is imaged with a medium-energy collimator and using the subtraction method described above to subtract the 67-Ga images from the 111-In images. At 4 hrs, and then improving by 6 hrs., a positive image of the left inguinal node is noted while the right inguinal node is virtually negative.

EXAMPLE 7

Organ Scintigraphy

Hybridoma-monoclonal antibodies are made in the mouse to the Langerhans cells of the endocrine pancreas, derived from a human autopsy specimen shortly after death. The monoclonals reactive against the antigen epitopes showing relatively high specificity for Langerhans cells of the pancreas, as demonstrated, e.g., by immunohistology, are labeled with a gamma-emitting isotope, such as with I-131, and injected, e.g., Ø.15 mg monoclonal against endocrine pancreas antigen, labeled using Chloramine-T with I-131, at a dose of 1.Ø mCi, injected i.v. in a 3-month old male suspected of having pathology of the endocrine pancreas. External gamma-camera imaging is performed at 24, 48, 72, and 96 hours after injection, without subtraction. In this specific case, decreased to almost absent accretion of I-131 radioactivity in the pancreas is suggestive of endocrine pancreas pathology in an infant presenting with pancreas hormone deficiency shortly after birth.

Claims

1. The use of an antibody or antibody fragment which specifically binds a marker produced by or associated with normal cells or tissues of an organ, the antibody or antibody fragment being labelled with a radioisotope capable of external detection or with a magnetic resonance imaging enhancing agent in the preparation of material for use in a method of organ imaging in a mammalian subject by scintigraphy or magnetic resonance imaging, comprising the steps of:
   (a) parenterally injecting a mammalian subject at a locus and by a route providing access to said organ with the said labelled antibody or antibody fragment, the amount of the labelled antibody or antibody fragment being sufficient to permit a scintigraphic image or an enhanced magnetic resonance image of the organ to be obtained; and
   (b) obtaining a positive scintigraphic image or a positive enhanced magnetic resonance image of the organ at a time after injection of said antibody or antibody fragment sufficient for the antibody or antibody fragment to accrete in the organ and specifically bind to the marker; wherein a tumour or infectious lesion of said organ will be revealed as a negative scintigraphic or enhanced magnetic resonance image.

2. The use as claimed in claim 1, wherein the organ is a lymphatic structure and the antibody or antibody fragment specifically binds to normal lymphatic cells or tissues.

3. The use as claimed in claim 1 or claim 2, further comprising the additional steps of:
   (c) simultaneously or at an earlier or later time, parenterally injecting the subject, at the same or different locus and by the same or different route, with a further antibody or antibody fragment which specifically binds a marker produced by or associated with a tumour or infectious lesion, said further antibody or antibody fragment being labelled with a radioisotope capable of external detection or with a magnetic resonance image enhancing agent, the amount of the labelled further antibody or antibody fragment being sufficient to permit a positive scintigraphic image or a positive enhanced magnetic resonance image of the tumour or infectious lesion to be obtained;
   (d) obtaining a positive scintigraphic image or a positive enhanced magnetic resonance image of the tumour or infectious lesion, at a time after injection of the further labelled antibody or antibody fragment sufficient for the further antibody or antibody fragment to become specifically bound to the marker produced by or associated with the tumour or infectious lesion; and
   (e) subtracting the image obtained in step (b) of claim 1, which is a gross image including a positive image of normal tissues and cells of the organ and a negative image of any tumour or infectious lesion or accretion of a localised product thereof, from the image obtained in step (d) above, which is a positive image of the tumour or infectious lesion or accretion of a localised product thereof, to produce a refined positive image of the tumour, infectious lesion or accretion of a localised product thereof.

4. The use as claimed in claim 2 or claim 3, wherein said antibody or antibody fragment also specifically binds to at least one of normal liver and spleen tissue; wherein at least a portion of said antibody or antibody fragment

is injected by a systemic route; and wherein in step (b), said image includes an image of at least one of the liver and the spleen, and in step (e), said image of at least one of the liver and the spleen is also subtracted from the image obtained in step (d).

5. The use as claimed in any one of claims 1 to 4, wherein each said label is a radioisotope capable of external detection, and each said image is a scintigraphic image.

6. The use as claimed in any one of claims 1 to 4, wherein each said label is a magnetic resonance enhancing agent, and each said image is a magnetic resonance image.

7. A lymphographic imaging kit for imaging a tumour or infectious lesion or localised product thereof in a mammalian subject, comprising:
(a) at least one gross lymphoscintigraphic or lymphomagnetic resonance imaging agent which comprises an antibody or antibody fragment which specifically binds to normal lymphatic cells or tissues, said antibody or antibody fragment being labelled with a radioisotope capable of external detection or a magnetic resonance image enhancing agent (as a first reagent);
(b) at least one antibody or antibody fragment which specifically binds a marker produced by or associated with a tumour or infectious lesion, said antibody or antibody fragment being labelled with a radioisotope capable of external detection or with a magnetic resonance image enhancing agent (as a second reagent); and
(c) a sterile, pharmaceutically acceptable injection vehicle for concomitant injection of (a) and (b), or for separate injection of each of (a) and (b).

Patentansprüche

1. Verwendung eines Antikörpers oder Antikörperfragments, der bzw. das spezifisch eine durch normale Zellen oder Gewebe eines Organs erzeugte oder mit normalen Zellen oder Geweben eines Organs verbundene Markierungssubstanz bindet, wobei der Antikörper oder das Antikörperfragment markiert ist mit einem extern detektierbaren Radioisotrop oder mit einem Magnetresonanz abbildenden verstärkendem Mittel, bei der Herstellung von Material zur Verwendung in einem Verfahren zur Abbildung von Organen eines Säuger-Individuums mittels Szintigraphie oder Magnetresonanz-Abbildung, wobei die Verwendung die folgenden Stufen aufweist:
(a) parenterales Injizieren eines Säuger-Individuums an einem Locus und mittels eines Weges, der einen Zugang zu dem Organ eröffnet, mit dem markierten Antikörper oder Antikörperfragment, wobei die Menge an markierten Antikörper oder Antikörperfragment ausreichend ist zur Erziehung einer szintigraphischen Abbildung oder einer verstärkten Magnetresonanz-Abbildung des betreffenden Organs; und
(b) Erhalten einer positiven szintigraphischen Abbildung oder einer positiven verstärkten Magnetresonanz-Abbildung des Organs zu einer Zeit nach der Injektion des Antikörpers oder Antikörperfragments, die für den Antikörper oder das Antikörperfragment ausreichend ist, um im Organ zu adhärieren und spezifisch an die Markierungssubstanz zu binden; wobei ein Tumor oder eine infektiöse Schädigung des Organs als negative szintigraphische oder verstärkte Magnetresonanz-Abbildung angezeigt wird.

2. Verwendung nach Anspruch 1, wobei es sich bei dem Organ um eine lymphatische Struktur handelt, und der Antikörper oder das Antikörperfragment spezifisch an normale lymphatische Zellen oder Gewebe bindet.

3. Verwendung nach Anspruch 1 oder Anspruch 2, wobei die Verwendung die folgenden zusätzlichen Stufen aufweist:
(c) gleichzeitiges oder zu einem früheren oder späteren Zeitpunkt, parenterales Injizieren des Individuums an demgleichen oder einem unterschiedlichen Locus mittels des gleichen oder eines unterschiedlichen Weges mit einem weiteren Antikörper oder Antikörperfragment, der bzw. das spezifisch eine durch einen Tumor oder eine infektiöse Schädigung erzeugte oder mit einem Tumor oder einer infektiösen Schädigung verbundene Markierungssubstanz bindet, wobei der weiter Antikörper bzw. das weitere Antikörperfragment markiert ist mit einem extern detektierbaren Radioisotrop oder mit einem die Magnetresonanz-Abbildung verstärkenden Mittel, wobei die Menge an dem markierten weiteren Antikörper oder Antikörperfragment ausreichend ist zur Erzielung einer positiven szintigraphischen Abbildung oder einer positiven verstärkten Magnetresonanz-Abbildung des betreffenden Tumors oder der betreffenden infektiösen Schädigung;
(d) Erhalten einer positiven szintigraphischen Abbildung oder einer positiven ver-

stärkten Magnetresonanz-Abbildung des Tumors oder der infektiösen Schädigung zu einer Zeit nach der Injektion des weiteren markierten Antikörpers oder Antikörperfragments, die für den weiteren Antikörper oder das weitere Antikörperfragment ausreichend ist, um spezifisch an die durch den Tumor oder die infektiöse Schädigung erzeugte oder mit dem Tumor oder der infektiösen Schädigung verbundene Markierungssubstanz gebunden zu werden; und
(e) Subtrahieren der in Stufe (b) von Anspruch 1 erhaltenen Abbildung, die eine Grobabbildung einschließlich einer positiven Abbildung von normalen Geweben und Zellen des Organs und einer negativen Abbildung von jedem Tumor oder jeder infektiösen Schädigung oder Akkretion eines lokalisierten Produkts davon darstellt, von der in Stufe (d) oben erhaltenen Abbildung, die eine positive Abbildung da Tumors oder der infektiösen Schädigung oder der Akkretion eines lokalisierten Produkts davon darstellt, um eine verfeinerte positive Abbildung des Tumors, der infektiösen Schädigung oder der Akkretion eines lokalisierten Produkts davon zu erzeugen.

4. Verwendung nach Anspruch 2 oder Anspruch 3, wobei der Antikörper oder das Antikörperfragment auch spezifisch an wenigstens eines von normalen Leber- und Milzgewebe bindet; wobei wenigstens ein Teil des Antikörper oder Antikörperfragments mittels eines systemischen Weges injiziert wird; und wobei in Stufe (b) die Abbildung eine Abbildung von wenigstens einer von der Leber und der Milz umfaßt, und in Stufe (e) die Abbildung von wenigstens einer der Leber und der Milz auch von der in Stufe (d) erhaltenen Abbildung subtrahiert wird.

5. Verwendung nach einem der Ansprüche 1 bis 4, wobei jede der Markierungssubstanzen ein extern detektierbares Radioisotrop ist, und jede der Abbildungen eine szintigraphische Abbildung ist.

6. Verwendung nach einem der Ansprüche 1 bis 4, wobei jede der Markierungssubstanzen ein Magnetresonanz verstärkendes Mittel ist, und jede der Abbildungen eine Magnetresonanz-Abbildung ist.

7. Kit für lymphographisches Abbilden zur Abbildung eines Tumors oder einer infektiösen Schädigung oder eines lokalisierten Produkts davon in einem Säuger-Individuum, wobei der Kit aufweist:
(a) wenigstens ein Mittel zur groben lymphoszintigraphischen oder lymphomagnetischen Resonanz-Abbildung, wobei das Mittel aufweist einen Antikörper oder ein Antikörperfragment, der bzw. das spezifisch an normale lymphatische Zellen oder Gewebe bindet, wobei der Antikörper oder das Antikörperfragment markiert ist mit einem extern detektierbaren Radioisotrop oder einem Magnetresonanz-Abbildung verstärkenden Mittel (als erstes Reagenz);
(b) wenigstens einen Antikörper oder ein Antikörperfragment, der bzw. das spezifisch eine durch einen Tumor oder eine infektiöse Schädigung erzeugte oder mit einem Tumor oder einer infektiösen Schädigung verbundene Markierungssubstanz bindet, wobei der Antikörper oder das Antikörperfragment markiert ist mit einem extern detektierbaren Radioisotrop oder mit einem Magnetresonanz-Abbildung verstärkenden Mittel (als zweites Reagenz); und
(c) ein steriles, pharmazeutisch verträgliches Injektionsvehikel für gleichzeitige Injektion von (a) und (b) oder für separate Injektion von jeweils (a) und (b).

Revendications

1. Utilisation d'un anticorps ou fragment d'anticorps qui se lie spécifiquement à un marqueur produit par ou associé à des cellules normales ou des tissus normaux d'un organe l'anticorps ou fragment d'anticorps étant marqué avec un radio-isotope susceptible de détection externe ou avec un agent amplifiant l'imagerie par résonance magnétique dans la préparation d'un produit utilisable dans un procédé de visualisation d'organes chez un sujet mammifère par imagerie par scintigraphie ou par résonance magnétique, comprenant les étapes consistant à :
(a) injecter par voie parentérale à un sujet mammifère, à un site et par un voie permettant l'accès audit organe, ledit anticorps ou fragment d'anticorps marqué, la quantité de l'anticorps ou fragment d'anticorps marqué étant suffisante pour permettre l'obtention d'une image scintigraphique ou d'une image par résonance magnétique amplifiée de l'organe, et
(b) obtenir une image scintigraphique positive ou un image par résonance magnétique amplifiée positive de l'organe au bout d'une durée après l'injection dudit anticorps ou fragment d'anticorps suffisante pour que l'anticorps ou fragment d'anticorps s'accumule dans l'organe et se lie spécifique-

ment au marqueur, dans laquelle une tumeur ou une lésion infectieuse dudit organe est révélée sous forme d'image scintigraphique négative ou d'image par résonance magnétique amplifiée négative.

2. Utilisation selon la revendication 1, dans laquelle l'organe est une structure lymphatique et l'anticorps ou fragment d'anticorps se lié spécifiquement à des cellules lymphatiques normales ou à des tissus lymphatiques normaux.

3. Utilisation selon la revendication 1 ou la revendication 2, comprenant en outre les étapes supplémentaires consistant à :

(c) injecter au sujet par voie parentérale, simultanément ou à un instant antérieur ou postérieur, au même site ou à un site différent et par la même voie ou par une voie différente, un anticorps ou fragment d'anticorps supplémentaire qui se lie spécifiquement à un marqueur produit par ou associé à une tumeur ou une lésion infectieuse, ledit anticorps ou fragment d'anticorps supplémentaire étant marqué avec un radio-isotope susceptible de détection externe ou avec un agent amplifiant l'imagerie par résonance magnétique, la quantité de l'anticorps ou fragment d'anticorps marqué supplémentaire étant suffisante pour permettre l'obtention d'une image scintigraphique positive ou d'une image par résonance magnétique amplifiée positive de la tumeur ou de la lésion infectieuse,

(d) obtenir une image scintigraphique positive ou une image par résonance magnétique amplifiée positive de la tumeur ou de la lésion infectieuse, au bout d'une durée après l'injection de l'anticorps ou fragment d'anticorps marqué supplémentaire suffisante pour que l'anticorps ou fragment d'anticorps supplémentaire soit lié spécifiquement au marqueur produit par ou associé à la tumeur ou a la lesion infectieuse, et

(e) soustraire l'image obtenue dans l'étape (b) de la revendication 1, qui est une image globale comprenant une image positive des tissus normaux et des cellules normales de l'organe et une image négative de toute tumeur ou lésion infectieuse ou accumulation d'un produit localisé de celle-ci, de l'image obtenue dans l'étape (d) ci-dessus, qui est une image positive de la tumeur ou de la lésion infectieuse ou de l'accumulation d'un produit localisé de celle-ci, pour produire une image positive affinée de la tumeur, de la lésion infectieuse ou de l'accumulation d'un produit localisé de celle-ci.

4. Utilisation selon la revendication 2 ou la revendication 3, dans laquelle ledit anticorps ou fragment d'anticorps se lie spécifiquement également à au moins un tissu parmi le tissu hépatique normal et le tissu splénique normal, dans laquelle une partie au moins dudit anticorps ou fragment d'anticorps est injectée par une voie systémique, et dans laquelle, dans l'étape (b), ladite image comprend une image d'au moins un organe parmi le foie et la rate, et dans l'étape (c), ladite image d'au moins un organe parmi le foie et la rate est également soustraite de l'image obtenue dans l'étape (d).

5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle chaque marqueur est un radio-isotope susceptible de détection externe et chaque image est une image scintigraphique.

6. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle chaque marqueur est un agent amplifiant la résonance magnétique et chaque image est une image par résonance magnétique.

7. Trousse d'imagerie lymphographique pour visualiser une tumeur ou une lésion infectieuse ou un produit localisé de celle-ci chez un sujet mammifère, comprenant :

(a) au moins un agent d'imagerie lymphoscintigraphique ou par résonance lymphomagnétique globale qui comprend un anticorps ou fragment d'anticorps qui se lie spécifiquement à des cellules lymphatiques normales ou à des tissus lymphatiques normaux, ledit anticorps ou fragment d'anticorps étant marqué avec un radio-isotope susceptible de détection externe ou avec agent amplifiant l'imagerie par résonance magnétique (en tant que premier réactif),

(b) au moins un anticorps ou fragment d'anticorps qui se lie spécifiquement à un marqueur produit par ou associé à une tumeur ou une lésion infectieuse, ledit anticorps ou fragment d'anticorps étant marquée avec un radio-isotope susceptible de détection externe ou avec un agent amplifiant l'imagerie par résonance magnétique (en tant que second réactif), et

(c) un véhicule pour injection stérile, pharmaceutiquement acceptable, pour l'injection concomitante de (a) et de (b) ou pour l'injection séparée de chacun parmi (a) et (b).